Expression of Russian Wheat Aphid (Homoptera: Aphididae) Resistance in Genotypes of Tall Fescue Harboring Different Isolates of *Acremonium* Endophyte

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ABSTRACT Experiments were conducted to compare the expression of Russian wheat aphid, *Diuraphis noxia* (Mordvilko), resistance in 2 genotypes of tall fescue grass, *Festuca arundinacea* Schreb., harboring different isolates of the endophytic fungus *Acremonium coenophialum* Morgan-Jones & Gams. Aphids did not select endophyte-free over endophyte-infected tiller sections in laboratory tests. In a laboratory population growth test, aphid numbers declined for 2 d on all endophyte-free plants and then increased. Aphid mortality was 100% on all infected plants after 4 d. Thus, antibiosis resistance to *D. noxia* was not specific to tall fescue genotype or to *Acremonium* isolate. In a field plot, numbers of Russian wheat aphids were significantly higher on endophyte-free tall fescue. Using these results, and results of other studies involving *Acremonium*-enhanced resistance to *D. noxia* in tall fescue and other host grasses, the potential for using *D. noxia* to screen grass germplasm for *Acremonium* fungi is discussed.

KEY WORDS aphids, *Diuraphis noxia*, *Acremonium* fungi, grass, plant resistance

INCREASING RECOGNITION of the link between *Acremonium* fungal endophytes in grasses and insect resistance, and the potential for manipulating these mycosymbionts to enhance the ability of infected plants to resist insect attack, is reflected by several recent reviews (see Dahlman et al. 1991, Breen 1994, Clement et al. 1994, Popay and Rowan 1994, Rowan and Latch 1994). This interest began in the early 1980s when scientists in New Zealand associated the presence of *Acremonium lolii* Latch, Christensen, & Samuels in perennial ryegrass, *Lolium perenne* L., with enhanced resistance to the Argentine stem weevil, *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) (Prestidge et al. 1982). Since then, >40 species of insects have been reported to be adversely affected by the presence of *Acremonium* endophytes in grasses (Clement et al. 1994, Rowan and Latch 1994). This plant resistance is the result of secondary metabolites (alkaloids) produced by the endophytes in association with their hosts. Both insect deterrence and toxicity are involved with the production of endophyte-associated alkaloids (Dahlman et al. 1991).

The expression of insect resistance (antixenosis or antibiosis) depends on several factors, including but not limited to the host plant genotype or species, the *Acremonium* species and isolate, and the insect species or biotype involved (Christensen and Latch 1991, Kindler et al. 1991, Breen 1993, Clement et al. 1994). The results of studies with aphids illustrate the complexity of grass–endophyte–insect relationships. For example, Christensen and Latch (1991) reported that expression of resistance in endophyte-infected tall fescue, *Festuca arundinacea* Schreb., to bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Homoptera: Aphididae), is *Acremonium* species- or isolate-specific. Moreover, the response of Russian wheat aphid, *Diuraphis noxia* (Mordvilko), to *Acremonium*-infected tall fescue is seemingly dependent on host plant genotype and endophyte genotype (Kindler et al. 1991). Other species of aphids, namely *Metopolophium dirhodum* (Walker), *Sitobion fragariae* (Walker), and *Macrosiphum avenae* (F.) (Homoptera: Aphididae), are not adversely affected by grass endophytes (Johnson et al. 1985, Latch et al. 1985). Breen (1993) and Clement et al. (1994) provide detailed information on the differential effects of *Acremonium*-infected tall fescue and perennial ryegrass on different species of aphids.

This study investigated further the influence of host plant genotype and different *Acremonium* isolates on grass–endophyte–aphid relationships. Specifically, we present data on host preference and rate of population growth of *D. noxia* on 2 geno-

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types of tall fescue, each harboring a different isolate of *Acremonium coenophialum* Morgan-Jones & Gams. We also present the 1st field data on *D. noxia* population densities on endophyte-free and infected tall fescue. Using the results of this study and those of Clement et al. (1990), Kindler et al. (1991), Wilson et al. (1991), and Clement et al. (1992), we conclude by discussing the utility of using the Russian wheat aphid to assay for the presence of *Acremonium* fungi in grass germplasm collections.

Materials and Methods

Aphids were obtained from a laboratory colony reared on 'Steptoe' barley, *Hordeum vulgare* L., in a growth chamber at 22 ± 1°C and a photoperiod of 14:10 (L:D) h. The culture plants in 15-cm pots were replaced every 10–14 d to avoid aphid overcrowding. The colony was initiated from aphids collected in September 1988 from a *Hordeum* spp. germplasm nursery near Pullman, WA. Laboratory host preference and population growth tests were conducted in separate growth chambers maintained under similar conditions of temperature and photoperiod.

The plant material consisted of 2 genotypes (clones 80-88, 80-89), each represented by plants with and without *A. coenophialum.* These genotypes represent 2 of the 5 parental genotypes of 'Georgia' 5 tall fescue (Bouton et al. 1993). The endophyte-free vegetative propagules (clones) of each genotype were developed in 1988 at the University of Georgia by treating propagules with a systemic fungicide (propiconizole; Ciba-Geigy, Agric. Div., Greensboro, NC) (De Battista et al. 1990). By this method, the same genotype was obtained in both infected and uninfected states, making it possible to separate plant genotype from endophyte effects. Clones of both genotypes, with and without endophyte, were received at Pullman, WA, in February 1990, and vegetatively propagated in a greenhouse to provide experimental material for laboratory and field studies 10–15 mo later. Light microscopy (Welty et al. 1986) confirmed the presence or absence of endophyte in leaf sheaths of clones.

Isolation of Endophytes. Confirmation of different endophyte isolates in infected plants of each genotype was obtained by measuring the lengths of 40 conidia of each isolate. Previously, Christensen and Latch (1991) used conidial dimensions to differentiate between different isolates of *A. coenophialum* in tall fescue.

To isolate fungi from tall fescue, basal stem sections (1–2 cm) were disinfected in a 0.525% solution of sodium hypochlorite plus 1 drop of Tween-20 per 100 ml of disinfectant solution for 1 min, then placed in petri dishes containing potato dextrose agar (PDA) with streptomycin sulfate and penicillin G (0.10 g of each per 1 liter of PDA). Cultures were incubated at 22 ± 2°C on a laboratory bench (diffused fluorescent light, photoperiod 10–12:12–14 [L:D] h) for ≥3 wk.

Scanning electron micrographs were prepared from plugs of mycelia from cultures and fixed in 2.5% glutaraldehyde 2.0% paraformaldehyde (buffered in 0.1M Pipes buffer [pH 7.4]), dehydrated in an ethanol series, critical point-dried, and sputter coated with gold. Samples were examined, and lengths of conidia were measured using a Hitachi S-570 scanning electron microscope.

Host Preference Tests. Tests were conducted by scattering ≈200 apterous aphids, previously starved for 2 h, over tillers (4-cm sections) of endophyte-free and endophyte-infected tall fescue on water-moistened filter paper in plastic petri dishes (15 cm diameter). Each dish was sealed with Parafilm, and the number of resting and feeding aphids on each plant sample was recorded 18 h later. The aphids wandering around in the petri dish also were counted, but these counts were disregarded in the analysis.

Tests 1 and 2 determined the preference responses of aphids to endophyte-free and endophyte-infected tillers of genotypes 80-88 and 80-89, respectively. Each test was conducted with 6 petri dishes (replicates). In each petri dish, 6 tiller segments (3 from an endophyte-free and 3 from an infected plant of the same genotype) were arranged in 2 rows (3 segments per row), with alternating segments from endophyte-free and infected plants. Segments were 2.5 cm apart. This procedure was used to determine preference of *R. padi* (Johnson et al. 1985, Siegel et al. 1985) and *D. noxia* (Clement et al. 1992) for endophyte-free and endophyte-infected tall fescue and perennial ryegrass plant samples, respectively, after 18 h.

Population Growth Test. Fifteen ramets from both endophyte-free and endophyte-infected plants of both genotypes (60 total) were transplanted to potting soil in white plastic Supercells (Ray Leech Conetainers, Canby, OR) (3.8 by 20.6 cm) placed in holding racks positioned over trays filled with water in a growth chamber (22.2–26.7°C; photoperiod 14:10 [L:D] h); these were clipped to a uniform height of 3.5 cm, then fertilized with Peters 20:20:20 (N:P:K) (Grace, Fogelsville, PA) 9 d later. After 16 d, the most vigorously growing plants (n = 48) were selected for the experiment.

The experimental design was a randomized complete block with 12 replications; each block contained 1 endophyte-free and 1 endophyte-infected plant of each genotype (4 total). Fifteen adult apterous aphids were transferred with a camel's-hair brush to the base of each plant. Clear plastic tubes (3.6 by 30 cm), capped with nylon organdy screen, were tightly inserted into the Supercells to confine the aphids. The number of live aphids on each plant was recorded after 2 d and then daily for 5 d (6 censuses).

Field Study. Endophyte-free and endophyte-infected plants of genotype 80-89 were transplanted into the field in late May 1991 in 10 rows. The

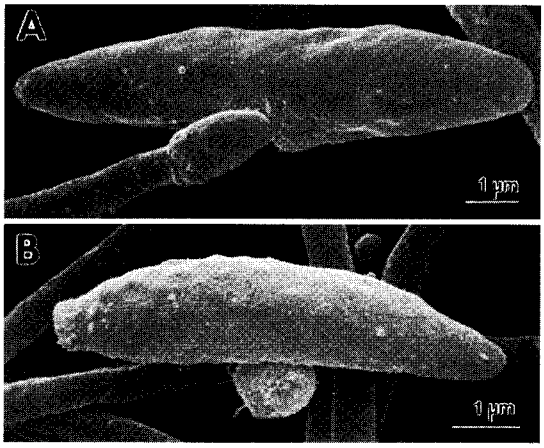

Fig. 1. SEM photomicrographs of conidia of *A. coenophialum* isolates from tall fescue genotypes 80-88 (A) and 80-89 (B).

experimental design was completely random with 5 replications of each treatment (row of endophyte-free or infected plants). Each 2-m row, or experimental unit, contained 5 plants. Inter-row and inter-plant spacings were 1.5 and 0.45 m, respectively. The plot was irrigated biweekly with overhead sprinklers delivering ≈20 mm per application over a 3-h period. Plants were clipped by hand to a height of 4–5 cm above the soil surface in early August to promote herbage growth.

To compensate for low aphid densities in the field, we placed 10-15 *D. noxia*-infested barley leaves from the laboratory colony in the center of each plant on 9 September 1991. The center 3 plants in each row were sampled 6 d later by carefully hand-clipping plants and placing herbage in a labeled plastic bag (40 by 31 cm). Bags were transported in cooled ice chests to a laboratory where the contents of each were immediately placed in a Berlese funnel for 72 h. Aphids that separated from herbage and remained in a bag were removed with a camel's-hair brush and preserved in a vial filled with 70% ethanol, along with the aphids extracted within the Berlese funnel. All *D. noxia* were counted.

Data Analyses. Data on dimensions of *Acremonium* conidia and aphid count data from the field test were analyzed by analysis of variance (ANOVA) (SAS Institute 1987). The observed frequency of aphids on endophyte-free and infected tillers in preference tests was compared with the expected ratio (50:50) using a replicated *G* test (Sokal and Rohlf 1981). Results of the population growth test were analyzed with SAS-GLM repeated-measures ANOVA (SAS Institute 1987, pp. 602–606). All data were transformed by $\log_{10}(x + 1)$ if zeros were present, to meet the normality and homogeneity of variance assumptions of ANOVA. Untransformed means are reported here.

Table 1. Distribution of *D. noxia* on stem sections of plants of tall fescue (genotype 80-89)

Replicate	Type of sample[a]	Observed frequency	G	df	Significance[b]
1	+	57	3.39	1	NS
	−	39	—	—	—
2	+	53	4.18	1	*
	−	34	—	—	—
3	+	27	2.19	1	NS
	−	39	—	—	—
4	+	31	3.30	1	NS
	−	47	—	—	—
5	+	63	9.53	1	**
	−	33	—	—	—
6	+	46	0.10	1	NS
	−	33	—	—	—
Pooled	—	—	3.44	1	NS
Heterogeneity	—	—	19.26	5	***
Total	—	—	22.71	6	***

NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[a] +, Endophyte-infected, −, endophyte-free.
[b] Expected distributions are based on 50:50 distribution of aphids on infected and endophyte-free stem sections.

Results and Discussion

Lengths (mean ± SEM) of conidia from endophytes isolated from host genotypes 80-88 and 80-89 ranged from 8.378 ± 0.173 to 5.902 ± 0.134 μm, respectively. These differences in conidial lengths (Fig. 1) are significantly different from one another ($F = 123.68$; df = 1, 78; $P = 0.0001$). The isolate from genotype 80-88 produced conidia that can be accommodated by the taxon *A. coenophialum*, which produces conidia 6.5–13 μm long (White and Morgan-Jones 1987). Because problems related to species definition of *Acremonium* fungi in tall fescue and other host grasses have not been resolved (White et al. 1993), the fungus from genotype 80-89 is considered to be a short conidia isolate of *A. coenophialum*.

In both preference tests, variable numbers of aphids settled on plant material after 18 h, generally much fewer than the ≈200 individuals initially released in each petri dish. Most aphids were wandering around petri dishes. This is reflected by the results of test 1 (Table 1). These results also show that aphid distributions were not significantly different ($P > 0.05$) between endophyte-free and endophyte-infected plant samples of genotype 80-89 in 4 of 6 replicates. The heterogeneity *G* test was significant ($P < 0.001$) because aphid distributions deviated significantly from a 50:50 expectation in 2 replicates (Table 1). This large heterogeneity *G* indicates that departures from expectation were not in a uniform direction; that is, aphids did not consistently prefer endophyte-free compared with endophyte-infected plant samples. In test 2, there was no evidence of aphid preference for 1 type of plant sample from genotype 80-88 (data not shown) (pooled *G* [0.408] and heterogeneity *G* [5.166] values were not significant ($P > 0.05$)).

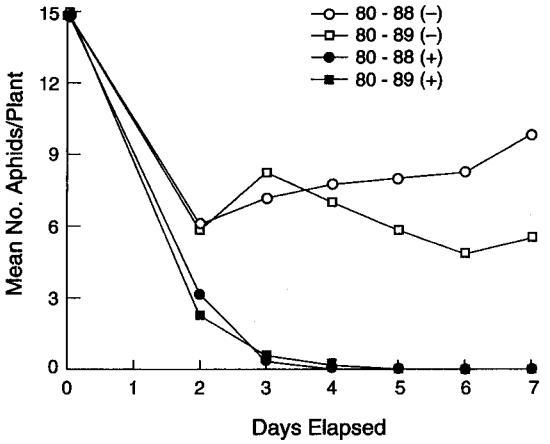

Fig. 2. Population trends of *D. noxia* on plants of 2 genotypes (80-88 and 80-89) of tall fescue, each represented by genetically identical plants, both infected (+) and free (−) of *Acremonium* endophyte.

In the population growth test, aphid numbers declined for 2 d on all endophyte-free plants before they stabilized and increased. No live aphids were found on infected plants of both genotypes after 4 d (Fig. 2). The repeated-measures analysis showed significant differences in *D. noxia* mortality among endophyte-free and endophyte-infected plants ($P = 0.0001$) and over time ($P = 0.001$) on these 2 plant types of both genotypes (Table 2). Aphid densities were not affected by the effects of plant genotype alone ($P = 0.316$). Also, there was a significant time × endophyte interaction (Table 2), indicating that the effect of time on aphid mortality on endophyte-free and endophyte-infected plants (irrespective of plant genotype) was different.

In the field plot, *D. noxia* averaged 32.4 ± 9.46 aphids on herbage from endophyte-free plants in 5 rows and 2.6 ± 0.90 on herbage from endophyte-infected plants in 5 rows. These mean values are significantly different from one another ($F = 15.15$; df = 1, 8; $P = 0.0046$).

The results show that the expression of antibiosis resistance in tall fescue to Russian wheat aphid was not specific to host plant genotype or to *Acremonium* isolate. Moreover, this antibiosis resistance was not accompanied by *D. noxia* deterrency to endophyte infection during 18-h preference tests. By contrast, *D. noxia* preferred endophyte-free over *A. coenophialum*-infected tall fescue in 48-h preference tests conducted by Kindler et al. (1991).

To date, *Acremonium*-enhanced resistance to *D. noxia* has been demonstrated in grass cultivars and lines of diverse taxonomic affinities, namely: 2 genotypes (80-88 and 80-89, this study) and 3 cultivars ('Arid', 'Forager', 'Mustang') of tall fescue (Clement et al. 1990, Kindler et al. 1991), two cultivars ('Repell', 'Regal') and 2 plant introduction lines (PI 462339, PI 205278) of perennial ryegrass (Clement et al. 1990, 1992; Kindler et al. 1991; Wilson et al. 1991), and six PI lines of annual ryegrass species (*L. persicum* Boiss & Hohen, PI 314446 and PI 222807; *L. rigidum* Gaudin, PI 287857 and PI 250805; *L. temulentum* L., PI 206691 and PI 249725) (S.L.C., D.G.L. and A.D.W., unpublished data). Collectively, this information suggests a potential for using *D. noxia* to assay collections of tall fescue and ryegrass germplasm for endophyte presence. However, because *D. noxia* was not adversely affected by 'Wrangler' tall fescue infected with an unidentified species of *Acremonium* endophyte (Kindler et al. 1991), *D. noxia* might not be sensitive to all grass–*Acremonium* combinations that could exist in germplasm collections. Therefore, more research is needed on the effect of different grass–*Acremonium* combinations on *D. noxia* behavior and survival to better understand its value as a screening agent for *Acremonium* fungi in grasses.

Although Latch et al. (1985) and Eichenseer and Dahlman (1992) proposed using *R. padi* as a rapid and inexpensive screen for endophytic fungi, its role in this regard may be more problematic because tall fescue infected with 5 *Acremonium* isolates exhibited no antixenotic or antibiotic activity

Table 2. Results of a repeated-measures ANOVA with mean number of *D. noxia* per endophyte-infected and endophyte-free plant of tall fescue (genotypes 80-88 and 80-89)

Source	df	Sum of squares	Mean square	F	*P*
Between					
Block	11	3.41	0.31	2.10	0.049
Genotype	1	0.15	0.15	1.04	0.316
Endophyte	1	35.67	35.67	241.86	<0.0001
Genotype × endophyte	1	0.08	0.08	0.55	0.466
Error	33	4.87	0.15	—	—
Within					
Time	5	2.74	0.55	23.84	<0.0001
Time × block	55	1.64	0.03	1.30	0.106
Time × genotype	5	0.24	0.05	2.13	0.065
Time × endophyte	5	2.67	0.53	23.27	<0.0001
Time × genotype × endophyte	5	0.21	0.04	1.84	0.107
Error	165	3.79	0.02	—	—

toward this aphid in a recent study (Christensen and Latch 1991).

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